A STIMULATOR FOR ANTIBODY PRODUCERS ISOLATED FROM SUPERNATANTS OF HUMAN BONE MARROW CELL CULTURES IN HEALTH AND DISEASE

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The study of mediators determining functioning of the immune system is currently receiving much attention. Some of these mediators, such as interleukins [8, 9], γ-interferon [15], macrophage migration inhibition factor [13], growth factors of T and B cells [11, 14], etc., are produced by immunocompetent cells after introduction of an antigen into the body whereas others are synthesized by cells of central lymphoid organs (thymus and bone marrow) continuously, irrespective of antigenic stimulation. These include, primarily, hormones synthesized by the reticuloendothelial stroma of the thymus. Cells of the other central organ of immunity, namely bone narrow, also synthesize various mediators which influence immune reactions.

The possibility that bone marrow cells may have a controlling influence on the immune response was first demonstrated in experiments with combined culture of lymph node cells from immune mice and bone marrow cells of intact animals [1, 3, 6]. It was found that bone marrow cells synthesize a mediator which can increase the number of antibody-producing cells in the lymph nodes of immunized animals by 2 or 3 times [4, 6]. This mediator was discovered in experiments on cultures of mouse bone marrow cells; later similar substances were isolated also from supernatants of bone marrow cell cultures from pigs, calves, hens, and other species of animals.

However, it still remained uncertain whether this mediator is synthesized by human bone marrow cells and, if it is, what is its biological activity in various diseases associated with bone marrow pathology.

The aim of this investigation was to isolate this mediator from supernatants of bone marrow cell cultures from healthy individuals and from patients with various lymphoproliferative diseases, and then to study the biological activity of this mediator.

EXPERIMENTAL METHOD

Bone narrow was obtained from donors by iliac puncture with a Kassirskii's needle followed by aspiration of the marrow. The bone marrow cells were mixed in culture medium 199 containing heparin, as stabilizer, in a concentration of 50 U/ml bone marrow suspension. Culture of the human bone marrow cells, isolation of the fraction containing the mediator stimulating antibody production from the supernatant of the bone marrow culture, immunization of animals, and the preparation of cell suspensions from the lymph nodes of immune animals were carried out by methods described previously [2, 5]. The protein concentration in the isolated fractions was determined by Lowry's method [12]. The number of antibody-producing cells was determined by Jerne's method [10] in the modification in [7].

The resulting cell suspensions from immune lymph nodes of (CBA × C56BL)F_1 mice were transferred to culture medium RPMI-1640 containing 20% embryonic calf serum, 200 mM glutamine, 0.5 M HEPES-buffer, and antibiotics (ampicillin and streptomycin) in a concentration of 0.3 mg/ml, and cultures in 96-well microplates with a concentration of 10^6 cells in 1 ml of culture medium (250 µl in each well for 24 h at 37°C and with 5% CO_2).

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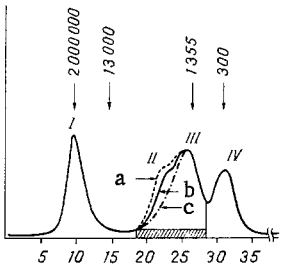

Fig. 1

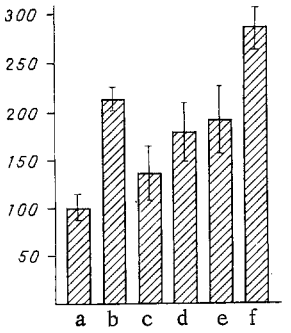

Fig. 2

Fig. 1. Gel-chromatography on Sephadex G-50 of supernatants of cultures of bone marrow cells obtained from healthy donors (b) and from patients with acute myeloblastic leukemia (c) and multiple myeloma (a). Abscissa, No. of tubes; ordinate, absorption spectrum in UV light at 280 nm. I) High-molecular-weight fraction (peak I); II, III) fraction from 5000 to 1000 daltons (peaks II and III); IV) phenol red (peak IV).

Fig. 2. Effect of stimulators of antibody producers obtained from bone marrow of healthy individuals and of patients with lymphoproliferative diseases on number of antibody-forming cells. Ordinate, number of antibody-forming cells per 10^6 lymph node cells of immune mice. a) Control; b) healthy subjects; c) acute myeloblastic leukemia; d) acute lymphoblastic leukemia; e) lymphosarcoma; f) multiple myeloma.

The fraction containing the stimulator of antibody producers was transferred into the wells in a dose of 15-20 μg (as protein) per 10^6 nucleated cells. The same eluting buffer as was used for fractionation of the supernatant of the human bone marrow cell culture by gel chromatography, was added to the control wells. The viability of the cells before and after culture was estimated by staining them with 0.1% trypan blue and counting the cells in a Goryaev's chamber. The degree of biological activity of the stimulator of antibody production from human bone marrow was judged from the ratio of the number of antibody-forming cells per 10^6 living nucleated cells in wells to which the stimulator of antibody producers was added to the number of antibody-forming cells in the control wells.

EXPERIMENTAL RESULTS

Bone marrow cells obtained from clinically healthy donors (6 cases) and from patients with acute myeloblastic leukemia (8), with acute lymphoblastic leukemia (10), lymphosarcoma (11), and multiple myeloma (14 cases) were cultured for 22-24 h. After the end of culture the supernatants of the bone marrow cell cultures were fractionated on a column filled with Sephadex G-50. The results are shown in Fig. 1. The fraction with stimulating activity was eluted in the region of the gel occupied by substances with molecular weights of 1000 to 5000 daltons, and which was evidently heterogeneous, for 2 or 3 peaks could be distinguished on the chromatogram (Fig. 1). The chromatographic profile of the active fractions isolated from the supernatant of human bone narrow cell cultures was virtually indistinguishable from that of the fraction isolated from the supernatant of a pig bone marrow cell culture and possessed antibody-stimulating activity (Fig. 2).

When the chromatographic fractions obtained from supernatants of bone marrow cell cultures from patients with multiple myeloma, from healthy subjects, and from patients with acute myeloblastic leukemia were compared, it was discovered that peak II was the most prominent peak on the chromatogram shown in Fig. 1a, it was weaker on that shown in Fig. 1b, and was virtually absent in the chromatogram in Fig. 1c.

On investigation of the antibody-stimulating activity of fractions isolated from supernatants of bone marrow cell cultures from healthy subjects and from patients with lymphoproliferative diseases such as acute myeloblastic leukemia, acute lymphoblastic leukemia, lymphosarcoma, and multiple myeloma it was found that the mediator synthesized by bone marrow cells from healthy subjects possessed the same stimulating activity as the corresponding

subatances isolated from supernatants of bone marrow cell cultures from animals of other species. For instance, when it was added to a culture of lymph node cells obtained at the peak of the secondary immune response, the number of antibody-forming producers in the culture was increased on average by 2.12 times. Meanwhile the stimulator of antibody production isolated from the supernatant of bone marrow cell cultures from patients with multiple myeloma increased the number of cells synthesizing antibodies by 2.79 times. Similarly fractions obtained from supernatants of bone marrow cell cultures from patients with acute myeloblastic leukemia has virtually no stimulating effect on the number of antibody producers in cell cultures from immune lymph nodes (coefficient of stimulation 1.36). Fractions obtained from the supernatants of bone marrow cell cultures from patients with acute lymphoblastic leukemia and lymphosarcoma, when added to a lymph node cell culture, were more active than fractions obtained from supernatants of bone narrow cell cultures from patients with acute myeloblastic leukemia, but less active than the fraction from bone marrow of healthy donors (Fig. 2). The results show that activity of the stimulator of antibody producers synthesized by bone marrow cells from patients with multiple myeloma was significantly higher than that from healthy individuals, whereas activity of the stimulator synthesized by bone marrow cells from patients with acute myeloblastic leukemia was lower. Activity of the stimulators of antibody producers, incidentally, correlated with the amplitude of peak II on the chromatograms (Fig. 1). It is possible that the fractions corresponding to peak II contain the stimulator of antibody production in a pure form. It is intended in the future to isolate and purify this peak and to estimate its antibody-stimulating activity.

The results thus demonstrate that human bone marrow cells, like bone marrow cells of other species of mammals and birds synthesize a mediator with the ability to increase the number of cells synthesizing antibodies in the productive phase of the immune response. When their activity was tested on the same test system, namely a culture of lymph node cells from immunized mice, similar results were obtained [4-6].

When the stimulator of antibody production was isolated from supernatants of bone marrow cell cultures from patients with multiple myeloma, its activity was much higher (Fig. 2). However, it is not yet clear whether the activity of this mediator was increased or whether there were more cells in the bone marrow of the patients with multiple myeloma which synthesize this mediator. Possibly the presence of foci of spontaneous immunoglobulin synthesis in the lymphoid organs of such patients is somehow connected with the increased activity of this mediator.

Low activity of the stimulator of antibody production from bone marrow of patients with acute myeloblastic leukemia (Fig. 2) was evidently due to the very small number of cells synthesizing this mediator in the bone marrow of these patients. However, the possibility likewise cannot be ruled out that this mediator is synthesized, but for some reason or another it is inactive.

In conclusion, a further study of this bone narrow mediator may help to shed light on the pathogenesis of certain lymphoproliferative diseases.

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